Korean J. Genetics 24 (1) : 21-29 (March 2002)
ⓒ The Genetics Society of Korea

Phylogenetic Analysis of Collembola Based on Highly Variable Region of 28S rRNA Gene Sequence

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Received October 22, 2001; accepted December 21, 2001

ABSTRACT

The rRNA gene of the *Willowsia bimaculata* (Entomobryidae) were determined and compared the D3 fragment of the 28S rDNA with those of other collembolan species for their phylogenetic relationships. Sequences were analyzed using maximum parsimony, maximum likelihood, and neighbor-joining methods. The inferred phylogenies support monophyly of the order Collembola. The present result agreed with the phylogeny of suborder Arthropleona divided into two superfamilies (Poduroidea and Entomobryoidea) as mentioned in conventional systematics and also when compared with analysis of data of their morphological and allozyme characters performed and published elsewhere by the present author. However, concerning the suborder Symphypleona phylogenetic position, MP and NJ analyses suggest possibility that the Symphypleona may be a sister of the superfamily Poduroidea. An overall review of the results shows that the fast evolving part of 28S rDNA are informative for determining affinities among close relatives such as the family level and partly genus level in Collembola.

Key words : *Willowsia bimaculata*, Collembola, Insecta, 28S rRNA gene, variable region, systematics, molecular phylogeny.

INTRODUCTION

Collembola are the most widespread terrestrial arthropods with largest range of ecological diversity. They are usually found in damp situations. Many species inhabit soil, leaf litter, decaying wood, fungi, mosses, caves, and even the surface of water. With the exception of a few species, Collembola are considered to be useful organisms whose role in organic decomposition is critical to the long-term well being of soils (Greenslade, 1991; Hopkin, 1997).

The classification and phylogenetic relationships among groups of the order Collembola have been a subject of disagreement among various authors without reaching a satisfactory consensus (Paclt, 1956; Salmon, 1964; Moen and Ellis, 1984; Soto-Adames, 1996; Bellinger et al., 2001). Comparative external morphology, however, is still the main approach used in collembolan taxonomy. The Collembola were traditionally divided into two suborders each characterized by a distinct body form, Arthropleona with elongate body and Symphypleona with globular body. The suborder Arthropleona was classified in two superfamilies, Poduroidea (Poduromorpha) with prothorax free and Entomobryoidea (Entotomobryomorpha) with prothorax more or less reduced.

Paclt (1956), however, was the first to consider Poduridae in Poduromorpha and Actaletidae in Entomobryomorpha as sister taxa based on the hypognathous position of the head and place these taxa as the sister group of Symphypleona which also share this character.

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The sister group relationship of Poduridae and Actaletidae to Symphypleona was subsequently supported by Salmon (1964). He defined suborder Metaxypleona for *Podura* and *Actaletes*. Moen and Ellis (1984) have suggested that Arthropleona and Poduromorpha should be discarded from formal classification because their definition is based on plesiomorphic characters and they may not form natural group. They considered a sister group relationship between Poduridae and Symphypleona-Neelipleona. Bellinger et al. (2001) divided the Collembola into five suborders, based on a linear phylogeny of the progressively increasing function of the furca. In the tree showing them, based on phylogenies proposed by Cassagnau (1971), Massoud (1976), Moen and Ellis (1984), Bretfeld (1986), Fjellberg (1994) and Soto-Adames (1996), Poduromorpha branched first, Metaxypleona next and Neelipleona as a sister group of Entomobryomorpha and Symphypleona.

During the past decade, the field of molecular systematics which used various molecular data to infer the phylogenetic relationships among taxa has been rapidly developed. Changes in gene sequences are thought to be largely unaffected by the environment. And the similarity of DNA sequences may accurately reflect relationships among taxa (Avise, 1994; Han, 2000; Jung, 2000). However a given gene may be informative at one taxonomic level, but uninformative at another (Hopkin, 1997).

Ribosomal RNA (rRNA) genes evolve at a relatively slow rate, which can provide optimal levels of sequence variation among higher taxonomic categories such as phyla, classes, and orders (Hwang et al., 1998; Min et al., 1998). Recently, the fast evolving parts of 18S and 28S rDNA have been used for the phylogenetic studies among close relatives at lower categories such as the family level in tiger beetles (Volger et al., 1997) and the genus level in Collembola (Carapelli et al., 1995).

Unfortunately, the molecular approach is in an early stage in Collembola systematics. I previously determined the 18S rDNA sequences from some Collembolan species (Lee et al., 1995a; 1995b). I found that the 18S rDNA sequence analyses of Collembola give contradictory results when the two different methods are used. They did not support taxonomic decision at family level. Recently, Frati and Dell'Ampio (2000) used the nuclear 28S rDNA and the mitochondrial COII gene to establish phylogenetic relationships among species of the family Neanuridae. The D3-D5 fragment of the rRNA gene was, however, very conserved and supplied little information of relationships at lower category. The phylogenetic reconstruction based on COII gene was partly in accordance with morphological data.

The sequence analysis of the rRNA gene have been attempted little in the species of the family Entomobryidae in contrast with the some information available on these genes in some other families. Therefore, the rDNA sequence of the *Willowsia bimaculata* in Entomobryidae was compared with the D3 fragment of 28S rDNA of 15 collembolan species in five families reported in the GenBank. It was hoped that the highly variable region of 28S rDNA might provide usefulness for reconstructing phylogenetic relationships within and among five collembolan families. This is the first attempt that phylogentic relationships among the families in Collembola are inferred by comparing the fragment of the 28S rDNA.

MATERIALS AND METHODS

Genomic DNA extraction

The preparation of the collembolan genomic DNA was carried out with slight modification of the method of Edwards et al. (1991). Fifty individuals of Collembola were crushed in a 1.5 ml microtube with liquid nitrogen. After adding 500 μl of DNA extraction buffer (100 mM Tris-HCl, pH 8.0; 80 mM EDTA, pH 8.0; 160 mM Sucrose) to each tube, they were mixed by inverting the tubes several times. SDS (10%) and proteinase K (final concentration 15 mg/ml) were added, mixed, and incubated overnight at 37°C. Then equal volume of chloroform and phenol were added. The tube was spun at 3,000 rpm for 5 min and the supernatant was transferred to a new tube to be mixed with two volume of cold absolute ethanol to precipitate the genomic DNA by centrifugation for 10 min at 3,000 rpm. The genomic DNA was dissolved with 20 μl of double-distilled water.

Cloning and sequencing

The sequences were obtained from cloned PCR fragments. A fragment of the rDNA cistron, including the ITS2, was amplified by polymerase chain reaction (PCR) and the primers were as follows: c58 (5′-GAACTGCAGGACACATGAACA-3′) and c280 (5′-CTGACCAGGCATAGTTCACC-3′). I designed forward and reverse primer sequences in the conserved coding region of the 5.8S and 28S rRNA genes based on known sequences of insects. The PCR reaction mixture comprised of 50 ng templete DNA, 25 pmol of each primer, 4 μl of 2.5 mM each dNTP, 5 μl of 10x reaction buffer, 0.5 unit of *Taq* DNA polymerase

Table 1. List of Taxa Used for Phylogenetic Comparisons.

Taxa		Species	Abbreviation	Accesion Number
Insecta				
Collembola				
Arthropleona				
Poduroidea				
Poduridae		*Podura aquatica*	*Poduaqua*	AF005468
Neanuridae	Friesenae	*Friesea grisea*	*Friegri*	AJ251739
	Pseudachorutinae	*Anurida maritima*	*Anurimar*	AJ251738
	Neanurinae	*Bilobella aurantiaca*	*Biloaura*	AJ251730
		Bilobella massoudi	*Bilomass*	AJ251732
		Neanura muscorum	*Neanmus*	AJ251733
Entomobryoidea				
Isotomidae		*Tetracanthella* sp.	*Tetracan*	AJ009851
		Isotomurus nebulosus	*Isomuneb*	AJ009850
		Isotomurus indipendente	*Isomuind*	X84953
		Isotomurus ghibellinus	*Isomughi*	X84957
		Isotomurus palustris	*Isomupal*	X84952
		Isotoma klovstadi	*Isotmakl*	Y11704
Entomobryidae	Orchesellinae	*Orchesella ranzii*	*Orchranz*	AJ009852
	Entomobryinae	*Willowsia bimaculata*	*Willbima*	AY066017
Symphypleona				
Sminthuridae		*Allacma fusca*	*Allacfus*	X84956
		Sminthurus viridis	*Sminvir*	AJ251741
Thysanura				
Lepismatidae		*Thermobia domestica*	*Therdome*	AF370808

(TaKaRa Ex Taq.). The PCR reaction was performed in a GeneAmp PCR System 2400 thermocycler (Perkin Elmer Co.). Thirty amplification cycles were performed, each involving denaturation at 94°C for 1 min, annealing at 60°C for 30 sec, and extention at 72°C for 1 min. The reaction was linked to a final polymerization step of 7 min at 72°C. The amplified products were extracted again with GeneClean II (Bio 101) and diluted in 20 μl of distilled water. The PCR products were cloned into the pGEM-T easy vector (Promega Co.) and transformed into *E. coli* JM109 cells. Double-stranded recombinant plasmid DNA was purified using a plasmid purification kit (Promega Co.). The amplified rDNA fragments sequenced with an ABI Prism 377 automatic DNA sequencer using BigDye terminator cycle sequencing kit V2.0 (Perkin Elmer Co.) in Korea Basic Science Institute, Kwangju Branch. Both directions were fully sequenced.

Data analysis

The taxa used in this study are listed in Table 1. The sequence data of D3 stem of 28S rDNA in *Willowsia bimaculata* has been submitted to the GenBank (accession number: AY066017). The D3 fragments of the 28S rDNA nucleotide sequences were retrieved from the GenBank detabase with the aid of a BLAST network server (Altchul et al., 1997). The sequence of *Thermobia domestica* (Thysanura) was employed as an outgroup for all analyses. Nucleotide sequences of 17 species were initially aligned using the Clustal W (Thomson et al., 1994) alignment program before performing the phylogenetic analysis. Sites with missing data or gaps were excluded from all analyses. After aligning, a set of sequences were confirmed to the format of the PHYLIP package ver. 3.5 (Felsenstein, 1993). From the realigned sequences, 1000 bootstrap samples were generated using the SEQBOOT program. The resulting matrices were subsequently subjected to the DNAPARS, DNAML and NEIGHBOR programs employing the maximum parsimony, maximum likelihood and neighbor joining analyses. Distance matrices were calculated using the DNADIST program and nucleotide substitutions (excluding gaps) were estimated using the Kimura's two parameter method (Kimura, 1980). A maximum likelihood tree was inferred using a transition/transversion ratio of 2 : 0. The trees were reduced to a single consensus tree using the CONSENSE program. Finally, a most probable phylogenetic

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                                                                                                       90
Therdome   ATTGGGTGTTTACGAAACCTAAAGGCGTAATGAAAGTAAAGTGTTTTCG-ATTACTTCGGTGGTCGAGAACCGAGGGAAGATGGAGTTTG  89
Poduaqua   ATTGAGAATGTT--AAACTCAAAGGCGTAATGAAAGTGAAGA---TCCA-ATTTTATGGAT--TTGAG----GAGAGATCATATCTTTTG  78
Anurimar   ATTGGAAATTACT-AAATCCAAAGGCATAATGAAAGTAAAGA---TCCA-ATTTTATGGAT--ATGAG----GAGAGATCATGGCTTTAG  79
Biloaura   ATTGGGAATTATT-AAACCCAAAGGCATAATGAAAGTAAAGA---TCCA-ATTTTATGGAT--ATGAG----GAGAGATCATGGCTTTAA  79
Bilomass   ATTGGGAATTACT-AAACCCAAAGGCATAATGAAAGTAAAGA---TCCA-ATTTTATGGAT--ATGAG----GAGAGATCATGGCTTTAA  79
Neanmus    ATTGGGAAATACT-AAACCCAAAGGCATAATGAAAGTAAAGA---TCCA-ATTTTATGGAT--ATGAG----GAGAGATCATGGCTTTAA  79
Friegi     ATTGGGAATTATT-AAACCCATAGGCATAATGAAAGTAAAGA---TCCA-ATTTTATGGAT--TTGAG----GAGAGATCATGGCTTTGG  79
Isomuind   ATTGGAAATGT---AAACCCAAAGGCATAATGAAAGTAAAGAA--TTCG-ATTGC--GAAT--TTGAG----GAGAGATCAAGGGCAGAG  76
Isotmakl   ATTGGGAATGT---AAACCCAAAGGCATAATGAAAGTAAAGAA--TTCG-ATTGC--GAAT--TTGAG----GAGAGATCAAGGGCAGAG  76
Isomuneb   ATTGGGAATGT---AAACCCAAAGGCATAATGAAAGTAAAGAA--TTCG-ATTGC--GAAT--TTGAG----GAGAGATCAAGGGCAGAG  76
Isomughi   ATTGGGAATGT---AAACCCAAAGGCATAATGAAAGTAAAGAA--TTCG-ATTGC--GAGT--TTGAG----GAGAGATCAATGATAGAG  76
Isomupal   ATTGGGAATGT---AAACCCAAAGGCGGAATGAAAGTAAAGAA--TTCG-ATTGC--GAGT--TTGAG----GAGAGATCAATGATATAG  76
Tetracan   ATTGGGAAAGT---AAACCCAAAGGCATAATGAAAGTAAAGAA--TTCG-ATTGC--GAAT--TTGAG----GAGAGATCATGGAT-GAG  75
Orchranz   ATTGGGAATGA---AAACCCAAAGGCGTAATGAAAGTGAAGA---TTCG-ATTGC--GAAT--TTGAG----GAGAGATACCGTAGCGGT  75
Willbima   ATTGGGAATGA---AAACCCAAAGGCGTAATGAAAGTGAAGA---TTCG-ATTGC--GAAT--TTGAG----GAGAGATCCTGGCTCTGT  75
Allacfus   ATTGAGAATGTA--AAACTCAAAGGCATAATGAAAGTGAAGAT--TCGT-CTTGTACGGAT---TGAG----GAGAGATCATGTCAATAC  78
Sminvir    ATTGAGAATGTA--AAACTCAAAGGCATAATGAAAGTGAAGA---TCCGTCTTGCACGGAT---TGAG----GAGAGATCATGGCAATAC  78
                                                                                                       180
Therdome   TCAAGTTTGTTCTGATCCTTTTCGGAGGTGAGGTCGGGCTTGGCAACTTGATCCGAGTCAATTTGGCAAAGGATACTCCCGCACTCCCAG  179
Poduaqua   TT----------TAGTA--TTCCGTA----AGGT-----TT----ATTAAAAAAA-----GTATG--------------CGCATCTCCTG  124
Anurimar   ATCT--------TAATA--TTCTGAA----AAGT-----TT----ATTAAGTAAA-----GTATG--------------CGCATCTCCTG  127
Biloaura   GTCT--------TGATA--TTCCGTA----AGGT-----TT----ATTGAGTAAA-----GTATG--------------CGCATCTCCTG  127
Bilomass   GTCT--------TGATA--TTCCGTA----AGGT-----TT----ATTGAGTAAA-----GTATG--------------CGCATCTCCTG  127
Neanmus    GTCT--------TGATA--TTCCGTA----AGGT-----TT----ATTGAGTAAA-----GTATG--------------CGCATCTCCTG  127
Friegi     A-CT--------TGATA--TATTGAA----AGAT-----TT----ATTGAGTAAA-----GTATG--------------CGCATCTCCTG  126
Isomuind   A-----------GGGGG--TTTCGTA----AGAC-----TC----ACTGTCTCTG-----TTTTG--------------CGCATCTCCTG  121
Isotmakl   A-----------TGGGG--TTTCGTA----AGAC-----TC----ACTGTCTCTG-----TTTTG--------------CGCATCTCCTG  121
Isomuneb   A-----------TGGGG--TTTCGTA----AGAC-----TC----ACTGTCTCTG-----TTTTG--------------CGCATCTCCTG  121
Isomughi   A-----------TGGGG--TTCCGTA----AGGT-----TC----AATGTCTCTG-----TTTTG--------------CGCATCTCCTG  121
Isomupal   A-----------TGGGG--TTCTGTA----AAGA-----TC----ACTGTCTATG-----TTATG--------------CGCATCTCCTG  121
Tetracan   AG----------TGGGA--TTCCGCA----AGTT-----TT----CCGCTCT-TG-----TTATG--------------CGCATCTCCTG  120
Orchranz   GTGG--------TGGC---TTCCGTA----AGGT-----T-----GTTGCCACAGC----TTGGT--------------CGCATCTCCGG  122
Willbima   T-----------TGGCA--TTCCGTA----AGGT-----TT----GCTGGGCAGG-----GTGGG--------------CGCATCTCCGG  120
Allacfus   T-----------TGGCA--TTCCGTA----AG-T-----TT----GCTGGGTAAATT---GTATG--------------CGCATCTCCTG  124
Sminvir    T-----------GGGCA--TTCCTAA----AGGT-----TT----GCTCGGTACTTT---GTATG--------------CGCATCTCCTG  125
                                                                                                263
Therdome   GGCGTCTCGTTCTCATTGCGAGATGAGGCGCACCTAGAGCGTACACGTTGGGACCCGAAAGATGGTGAACTATGCCCGGCCAG  262
Poduaqua   GACGTCTT-AAC-CTTCGA-AGGTGGGGCGTACCTAGAGCATACACGTTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAG  204
Anurimar   GATGTCTT-AAC-CTCTGA-AGGTAGGGCATACCTAGAGCATACACGTTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAG  207
Biloaura   GATGTCTT-AAC-CTCTGA-AGGTAGGGCATACCTAGAGCATACACGTTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAG  207
Bilomass   GATGTCTT-AAC-CTCTGA-AGGTAGGGCATACCTAGAGCATACACGTTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAG  207
Neanmus    GATGTCTT-AAC-CTCTGA-AGGTAGGGCATACCGAGAGCATACACGTTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAG  207
Friegi     GACGTTTT-AAC-CTTTGA-AGGTAGGGCGTACCTAGAGCATACACGTTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAG  206
Isomuind   GACGTCTA-TAC-CTCTGA-AGGTTGGGCGTACCAAGAGCATATACGTTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAG  201
Isotmakl   GACGTCTA-TAC-CTCTGA-AGGTTGGGCGTACCAAGAGCATATACGTTGGGACCCGAAAGGTGGTGAACTATGCCTGATCAG  201
Isomuneb   GACGTCTA-TGC-CTCTGA-AGGTTGGGCGTACCAAGAGCATATACGTTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAG  201
Isomughi   GACGTCTA-TAC-CTCTGA-AGGTTGGGCGTACCAAGAGCATATACGTTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAG  201
Isomupal   GACGTCTA-TAC-CTCTGA-AGGTTGGGCGTACCAAGAGCATATACGTTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAG  201
Tetracan   GACGTCTAATAC-CTCTGA-AGGTTGGGCGTACCAAGAGCATACACGTTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAG  201
Orchranz   GACGTCTA-TAC-CTCTGA-AGGTAGGGCGTACCTAGAGCGTACACGTTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAG  202
Willbima   GACGTCTA-TAC-CTCTGA-AGGTGGGGCGTACCTAGAGCGTACACGTTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAG  200
Allacfus   GACGTCTA-TAC-CTCTGA-AGGTTGGGCGTACCTAGAGCATACACGTTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAG  204
Sminvir    GACGTCTA-TAC-CTCTGA-AGGTGGGGCGTACCTAGAGCATACACGTTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAG  205
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Figure 1. Sequence alignment of the D3 fragment of 28S rDNAs from 16 species of Collembola including *Thermobia domestica* (Thysanura) as an outgroup (- indicates alignment gap).

tree in each analysis was produced using the Treev32.

RESULTS AND DISCUSSION

The rDNA nucleotide sequence of the *Willowsia bimaculata* was determined and aligned with the D3 fragment of 28S rDNA sequences of 15 Collembolan species in five families and one Thysanuran species as an outgroup. The nucleotide sequences of the 16 species except *Willowsia bimaculata* were obtained from the data of GenBank (Table 1). The total aligned set of nucleotides of the 17 species is shown in Figure 1. The aligned sequence length over all taxa is 263 bp. The multiple alignment of these 17 taxa showed that the sequence of Thysanuran species was longer than those of Collembolan species. In overviewing 17 sequencies, 1 bp insertion at 50 (T) site and 47 bp deletions at 14 (G), 69-72 (AACC), 95-102 (GTTTGTTC), 108-109 (CT), 117-120 (GGTG), 125-129 (CGGGC), 132-135 (GGCA), 148-150 (TCA), 156-169 (GCAAAGGATACTCC), 193 (T) and 200 (G) sites were noted in those of Collembolan species.

In order to avoid the potential risks and inconsistencies of a single method or data set, the phylogenetic reconstructions were based on three different approaches, methods of maximum parsimony (MP), maximum likelihood (ML) and neighbor joining (NJ). The inferred phylogenies support monophyly of the Order Collembola (bootstrap value: 100%). In the phyletic trees of MP (Fig. 2) and ML (Fig. 3) the orders of branch within each family were same, but the position of Symphypleona were inconsistent in the two methods. In the MP tree, Collembola were subdivided into two branches: Subtribe 1 clustered with Sminthuridae which includes *Allacma fusca* and *Sminthurus viridis*, Poduridae which includes *Podura aquatica*, and Neanuridae which includes *Friesea grisea, Bilobella aurantiaca, Bilobella massoudi, Neanura muscorum* and *Anurida maritima*, whereas Subtribe 2 clustered with Isotomidae which includes *Isotoma klovstadi, Tetracanthella sp.*, and 4 species of *Isotomurus* (*I. nebulosus, I. indipendent, I. ghibellinus* and *I. palustris*), and Entomobryidae which includes *Orchesella ranzii* and *Willowsia bimaculata*. In the ML tree, Collembola were also divided into two subtrees but the position of Symphypleona was different from that of MP tree: One contains Poduridae and Neanuridae, the other clade includes Isotomidae and Entomobryidae plus Symphypleona. The phylogenetic reconstruction based on NJ (Fig. 4) was partly in accordance with MP and ML

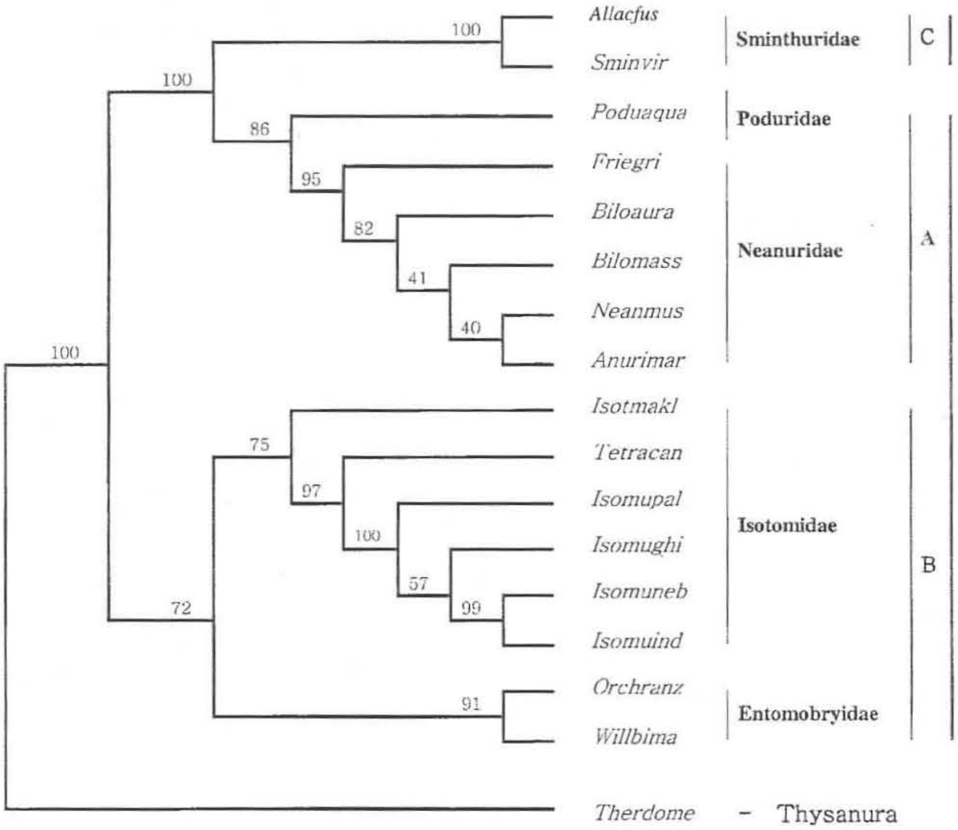

Figure 2. The most parsimonious tree using DNAPARS in PHYLIP package. The numbers above the branches are confidence levels of consensus tree from 1000 bootstrap replicates (A: Poduroidea, B: Entomobryoidea, A+B: Arthropleona, C: Symphypleona, A+B+C: Collembola).

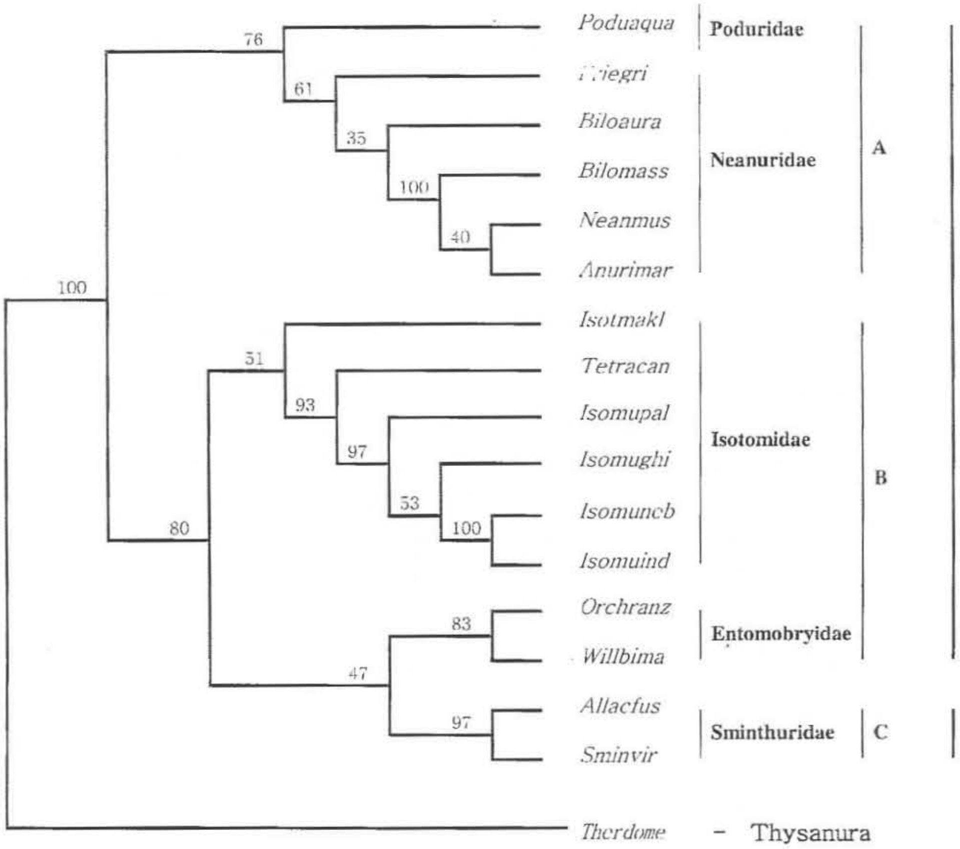

Figure 3. The maximum likelihood tree using DNAML in PHYLIP package. The numbers above the branches are confidence levels of consensus tree from 1000 bootstrap replicates (A: Poduroidea, B: Entomobryoidea, A+B: Arthropleona, C: Symphypleona, A+B+C: Collembola).

analyses, but it was disaccordant for the placement of some species. *Isotoma klovstadi* was a sister taxon to the Entomobryoidea clade, instead of ingroup to the taxa of Isotomidae. However, because of the lower bootstrap value (43%), the position of it is not obvious.

The clustering pattern of present results agreed with the phylogeny of suborder Arthropleona divided into two superfamilies of Poduroidea and Entomobryoidea as mentioned in conventional systematics and also when compared with analysis of data of their morphological and allozyme characters (Park et al., 1996) performed and published elsewhere by the present auther. In cladistic analysis of morphological characters and allozyme analysis using four species representing each family of the superfamily Poduroidea and three species of families of the superfamily Entomobryoidea, Arthropleona were subdivided into two branches. However, the 18S rDNA sequence analysis yielded only a limited support to this scheme. The NJ tree based on 18S rDNA sequence data supported clearly Poduroidea grouping but not the Entomobryoidea one. The MP method, however, did not support monophyly of the superfamily Poduroidea but suggested the single grouping of three families of Entomobryoidea. The 18S rDNA sequence analyses gave contradictory results when the two different methods were used. These results showed the primary sequence from 18S rRNA gene is not suitable for determining taxonomic status at lower categories such as the family level in Collembola, or suggest that two superfamilis of Collembola may not form natural groups. In present study, the relationship among two superfamily groups is obvious, and the clustering pattern of the present molecular dada analyses agreed with the traditional view of these two superfamilies. The taxonomic position of Poduridae is by no mean certain as it shares some characteristics with the Arthropleona and others with Symphypleona and Neelipleona (Hopkin, 1997). Frish (1978) found that the heart of Poduromorpha, including *Podura*, has six ostia, and in Symphypleona the ostia are reduced to

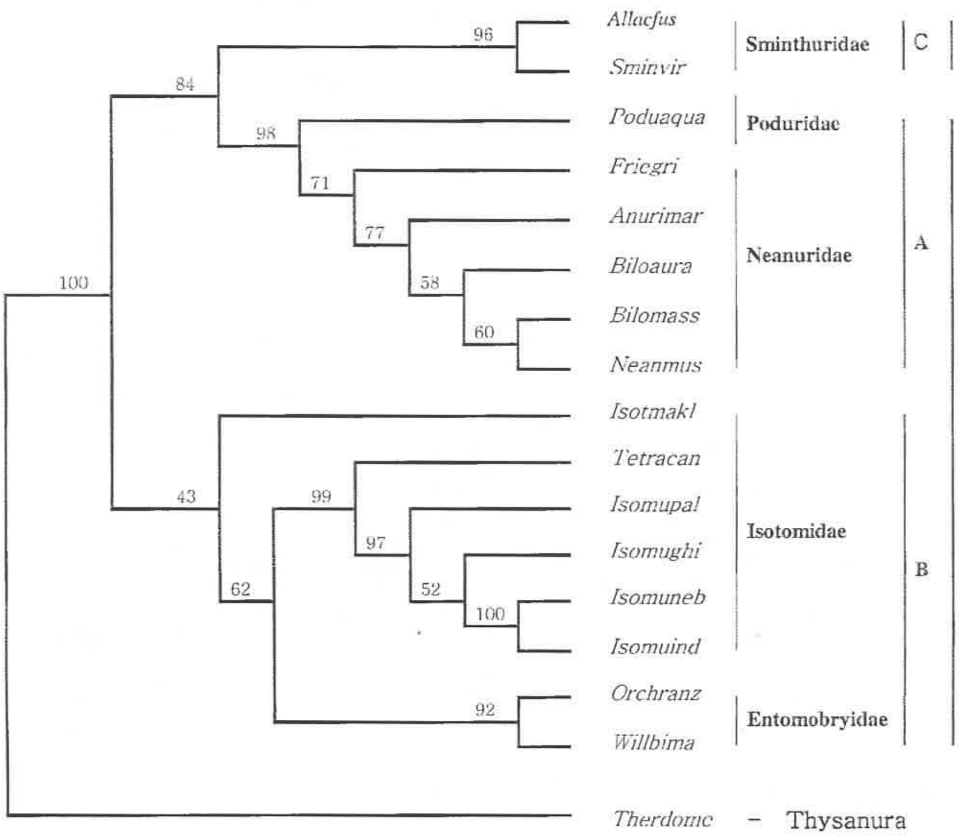

Figure 4. The neighbor joinning tree using NEIGHBOR in PHYLIP package. The numbers above the branches are confidence levels of consensus tree from 1000 bootstrap replicates (A: Poduroidea, B: Entomobryoidea, A+B: Arthropleona, C: Symphypleona, A+B+C: Collembola).

three. According to Hemmer (1990), the presence of a haploid chromosomes number in *P. aquatica* of n = 11 compared to typically n = 5 in Symphypleona makes a close relationship between these two taxa questionable (Hopkin, 1997). Based on these Frish (1978) and Hemmer (1990) concluded that the characters invoked by Paclt (1956) and Salmon (1964) to relate Poduridae and Symphypleona represented convergence and not real homology. The present results based on 28S rDNA analysis, even though other species of *Podura* were not included, strongly suggests that this species show greater affinity to Neanuridae (Poduromorpha, Arthropleona) than to Symphypleona. This result supports the perspective of Bellinger et al. (2001) based on a linear phylogeny of the progressively increasing function of the furca.

Although the Collembola was traditionally classified in two suborders, Arthropleona and Symphypleona, the phylogeny have been a subject of disagreement among various authors. Soto-Adames (1996) divided it into four groups (Poduromorpha, Entomobryomorpha, Symphyplona, and Neelipleona), while Bellinger et al. (2001) classified it in five groups (including Metaxypleona). And several major questions concern the relatedness among suborders have been present. Some authors regarded the Symphypleona as more closely related with the Poduridae (Paclt, 1956; Salmon, 1964; Moen & Ellis, 1984), whereas others considered closer to the Entomobryomorpha (Soto-Adames, 1996; Bellinger et al., 2001). We obtained contradictory results in the position of the suborder Symphypleona when the three different methods are used. Close affinity of Symphypleona and Poduridae resulting from the MP and NJ methods partly support the systems of Paclt (1956), Salmon (1964) and Moen and Ellis (1984). The relationship of them is strongly supported by bootstrap values (100% and 84%). However, the affinity of Symphypleona and Entomobryidae resulting from the ML method supports the systems of Soto-Adames (1996) and Bellinger et al. (2001). However, because of the lower bootstrap value (47%) of Entomobryidae and Sminthuridae, the relationship among them is not obvious. Therefore, the position of the Symphypleona is unstable throughout the three methods examined and requires more extensive examination to confirm its exact taxonomic position. However, MP and NJ analyses suggest possibility that Symphypleona may be a sister of the Poduromorpha.

These results show the molecular evidence of highly variable region of 28S rRNA gene sequence are informative for determining affinities among the family level in Collembola. However, the relationships of subfamilies or genus within Neanuridae are not clear and the arrangement presented here may not be a natural one. Particularly the relationships among the Pseudachorutinae and Neanurinae were uncertain. In the Isotomidae clade, *Isotoma* branched first and *Tetracanthella* next as a sister group to the taxa of *Isotomurus*. The relationships of genus within Isotomidae are obvious. These results show the molecular evidence of the fast evolving part of 28S rRNA gene sequence might partly provide usefulness for determining affinities among close relatives such as the genus level in Collembola.

It is universally accepted that the class Collembola is monophyletic group. However, classification schemes still use Linnaean categories which may be difficult to adapt to a stictly cladistic analysis of evolutionary relationships. The classification should be regarded for the moment as a convenient arrangement rather than the most parsimonious solution to the problem of how to subdivide the order Collembola (Hopkin, 1997). Several attempts have been made to reconstruct the evolution of Collemobola (Maynard, 1951; Yosii, 1961; Cassagnau, 1971; Uchida, 1971; Massoud, 1976). Nevertheless, a convincing phylogenetic tree has not yet been established. Obviously I need more sequences data of other collembolan and related species for the full picture of the evolution of Collembola.

ACKNOWLEDGEMENTS

I am grateful to Dr. Suhn-Young Im at Chonnam National University for her valuable technical assisstence in DNA sequencing experiment and Dr. Ui Wook Hwang for his help to data analyses. I thank Dr. Byung-Hoon Lee at Chonbuk National University and Dr. Keesook Lee at Chonnam National University for a critical reading of the manuscript.

REFERENCES

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W and Lipman J (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25: 3389-3402.

Avise JC (1994) Molecular markers, natural history and evolution. Chapman and Hall, London.

Bellinger PF, Christiansen KA and Janssens F (2001) Checklist of the Collembola : Collembola.

Bretfeld G (1986) Phylogenetic systematics of the higher taxa of Symphypleona Börner, 1901 (Insects, Entognatha, Collembola). In R. Dallai (ed.) Proc. 2nd Intern. Sem. Apterygota. Univ. of Siena, Siena, Italy. pp. 307-311.

Carapelli A, Frati F, Fanciulli PP and Dallai R (1995) Genetic differentiation of six sympatric species of *Isotomurus* (Collembola, Isotomidae); Is there any difference in their microhabitat preferences? Eur. Soil Biol. 31: 87-99.

Cassagnau P (1971) La phylogénie des Collemboles à la lumiére des structures endocrines rétrocérébrales. I. Sym. Intern. Zoolfilog. pp. 333-349, Salamanca Univ.

Edwards K, Johnstone C and Thomson C (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Res.* 19: 1-1349.

Felsenstein J (1993) PHYLIP (Phylogeny Inference Package), Version 3.5c, Department of Genetics, University of Washington, Seattle.

Fjellberg A (1994) The systematic position of the monotypic family Isotogastruridae (Collembola) with description of *Isotogastrura coronata* n. sp. From Fuerteventura, Canary Islands. Misc. Zool. 17: 123-127.

Frati F and Dell'Ampio (2000) Molecular phylogeny of three subfamilies of the Neanuridae (Insecta: Collembola) and the position of the Antarctic species *Friesea grisea* Schaeffer. Pedobio. 44: 342-360.

Frish K (1978) Das Herz der Collembola (Insecta)- Ein Beitrage zur Anatomie der Collembola. Zool. Anz. 201: 177-198.

Greenslade P (1991) Collembola (springtails); in *the Insects of Australia* (2nd ed.), CSIRO (ed.), Vol.1, pp. 252-264, Carlton: Melbourne Univ. Press.

Han SH, Jung YH, Ko MH, Oh YS, Koh SC, Kim MH and Oh MY (2000) Phylogenetic relationship between *Dendropanax morbifera* Léveille and *D. trifidus* Makino (Araliaceae) based on nuclear ribosomal DNA internal transcribed spacer (ITS) sequences. Korean J. Genetics 22(4): 257-264.

Hemmer W (1990) Karyotype differentiation and chromosomal variability in springtails (Collembola, Insecta). Biology and Fertility of Soils 9: 119-125.

Hopkin SP (1997) Biology of the Springtails; in *Insecta: Collembola*, pp. 330, Oxford Univ. Press.

Hwang UW, Kim W, Tautz D and Friedrich M (1998) Molecular phylogenetics at the Felsenstein zone: Approaching the Strepsiptera problem using 5.8S and 28S rDNA sequences. Mol. Phylogenetics Evol. 9: 470-480.

Jung YH, Han SH, Oh YS, Ko MH, Koh SC, Kim MH and Oh MY (2000) Genetic variation and phylogenetic analysis of genus *Abies* distributed in Korea based on RAPD and nuclear rDNA ITS2 sequences. Korean J. Genetics 22(4): 341-354.

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16: 111-120.

Lee BH, Hwang UW, Kim W, Park KH and Kim JT (1995a) Phylogenetic study of the suborder Arthropleona (Insecta: Collembola) based on morphological characters and 18S rDNA sequence analysis. Polskie Posma Entomol. 64: 261-277.

Lee BH, Hwang UW, Kim W, Park KH and Kim JT (1995b) Systematic position of cave Collembola, *Gulgastrura reticulosa* (Insecta) based on morphological characters and 18S rDNA nucleotide sequence analysis. Mémoires de Biospéol. 22: 83-90.

Massoud Z (1976) Essai de synthése sur la phylogénie des Collemboles. Rev. Ecol. Biol. Sol. 13: 241-252.

Maynard EA (1951) A monograph of the Collembola or Springtail Insects of New York State. 339 pp, Comstock Publ. Comp., Inc. Cornell Univ. Ithaca, New York.

Min GS, Kim SH and Kim W (1998) Molecular phylogeny of Arthropods and their relatives polyphyletic origin of Arthropodization. Mol. Cells 8: 75-83.

Moen P and Ellis WN (1984) Morphology and taxonomic position of *Podura aquatica* (Collembola). Entomol. Gener. 9: 193-204.

Paclt J (1956) Biologie der primar flugellosen Insekten. Jena, pp. 258.

Park KH, Kim JT and Lee BH (1996) Phylogeny and speciation of Entomobryoidea (Collembola, Insecta) from Korea. Kor. J. Syst. Zool. 12: 121-136.

Salmon JT (1964) An index to the *Collembola*, vol.I-II. Royal Soc. New Zeal., Wellington, Bull. 7: 1-644.

Soto-Adams FN (1996) in Maddison, D.R. et Maddison, W.P.

Thomson JD, Higgins DG and Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acid Res. 22: 4673-4680.

Uchida H (1971) Tentative key of the Japanese genera of Collembola in relation to the world genera of this order. I. Sci. Rep. Hirosaki Univ. 18: 64-76.

Volger AP, Welsh A and Hancock JM (1997) Phylogenetic analysis of slippage-like sequence variation in the V4 rRNA expansion segment in Tiger Beetles (Cicindelidae). Mol. Biol. Evol. 14: 6-19.

Yosii R (1961) Phylogenetische Bedeutung der chaetotaxie bei den Collembolen. Contr. Biol. Lab. Kyoto Univ. 25: 141-170.